Pestic. Sci. 1997, **51**, 375–382

Resistance Management Strategies in Malaria Vector Mosquito Control. A Large-Scale Field Trial in Southern Mexico*

Janet Hemingway,[1]† R. Patricia Penilla,[1] Americo D. Rodriguez,[1] Bronwen M. James,[1] William Edge,[1] Hilary Rogers[1] & Mario H. Rodriguez[2]

[1] School of Pure and Applied Biology, University of Wales Cardiff, PO Box 915, Cardiff CF1 3TL, UK
[2] Centro De Investigacion De Paludismo, Apartado Postal 537, Tapachula, Chiapas CP 30700, Mexico

(Received 29 March 1997; revised version received 18 June 1997; accepted 10 July 1997)

Abstract: A resistance management programme comparing rotations, mosaics and single use of insecticides for residual house-spraying against the insect vectors of malaria is being carried out in Southern Mexico. The area was chosen because of its prior history of insecticide use, relatively sedentary vector, and physical features of the area which limit inward migration of insects to the study area.

A high level of resistance to DDT and low levels of organophosphorus (OP), carbamate and pyrethroid resistance were detected by WHO discriminating-dose assays in field populations of *Anopheles albimanus* in the pre-spray period in the region where this resistance management project is being undertaken. After the first year of spraying, resistance, as measured by a discriminating-dose assay, was still at a high level for DDT and had risen for all the other insecticides.

Biochemical assays showed that DDT resistance was primarily caused by elevated levels of glutathione *S*-transferase (GST) activity leading to increased rates of metabolism of DDT to DDE. The numbers of individuals with elevated GST and DDT resistance were well correlated, suggesting that this is the only major DDT resistance mechanism in this population.

The carbamate resistance in this population was conferred by an altered acetylcholinesterase (AChE) mechanism. The level of resistance in bioassays correlated well with the frequency of individuals homozygous for the altered AChE allele. This suggests that the level of resistance conferred by this mechanism in its heterozygous state is below the level of detection of the bioassay.

The low levels of OP and pyrethroid resistance could be conferred by either the elevated esterase or monooxygenase enzymes. The esterases, however, are elevated only with *p*-nitrophenyl acetate (PNPA), and are unlikely to be causing broad-spectrum OP resistance. The altered AChE mechanism may also be contributing to the OP but not the pyrethroid resistance.

There were significant differences in some resistance gene frequencies for insects obtained by different indoor and outdoor trapping methods. To determine whether the different sampling methods were effectively sampling the same interbreeding population, RAPD analysis of insects obtained by different collection methods in different villages was undertaken. There was no observed variability in the RAPD patterns for the different mosquito samples with a number of primers.

Pestic Sci., **51**, 375–382, 1997
No. of Figures: 1. No. of Tables: 8. No. of Refs: 21

Key words: Anopheles, resistance management, malaria, rotations, mosquito, Mexico

* Based on a presentation at the Conference 'Resistance '97—Integrated Approach to Combating Resistance' organised by the Institute of Arable Crops Research in collaboration with the SCI Pesticides Group and the British Crop Protection Council and held at Harpenden, Herts, UK on 14–16 April 1997, and also presented in part at the Second Urban Entomology Congress, Edinburgh, UK in 1996.

† To whom correspondence should be addressed.

Contract grant sponsor: Insecticide Resistance Action Committee.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

1 INTRODUCTION

Resistance to many of the commonly used insecticides is a significant problem in control of medically important insects. The need to spray insecticides inside human habitations for malaria control severely restricts the number of compounds which can be used. The use of an insecticide until resistance becomes a limiting factor is rapidly eroding the number of available insecticides. A better management strategy may be the use of compounds in rotational or mosaic strategies.[1,2] Numerous mathematical models have been produced to determine the optimal strategies for resistance management in mosquito vector populations.[3–6] However, these models have not been tested before under field conditions due to the practical difficulties of accurately assessing the changes in resistance gene frequencies associated with different patterns of insecticide use.[7] With the advent of more sophisticated biochemical and molecular assays for resistance detection it is now practicable to accurately analyse large numbers of insects for a range of insecticide resistance genes and monitor their changes over time.[8] A large-scale field programme has now been established in Southern Mexico to determine whether there are any benefits for a three-compound annual rotation or two-compound spatial mosaic of insecticide use compared to blanket long-term use of a single insecticide. This paper reports the analysis of resistance gene frequencies in the major malaria vector *Anopheles albimanus* Weid. in the pre-spray and first annual post-spray periods. The initial data in this paper were originally reported at the International Urban Entomology Congress in Edinburgh in 1996.[9]

In choosing the region to undertake this project we deliberately picked an area where broad-spectrum resistance to a range of insecticides had developed two decades earlier and where resistance had since regressed (Table 1). The Chiapas region of Southern Mexico was formerly a region where moderate to high levels of agricultural insecticide were used around the mosquito breeding sites. In addition DDT has been used for malaria control in this region for more than 10 years and is still the insecticide of choice in Southern Mexico. The combined use of a range of different classes of insecticides for agricultural use and DDT for malaria control resulted in high levels of resistance to organochlorines, organophosphates, carbamates and pyrethroids in *An. albimanus* in the late 1970s. Since then a reduction in agricultural insecticide use in this region has resulted in regression of the resistance to a point where it is barely detectable using standard WHO bioassays (Table 1). In contrast the continued use of DDT for malaria control has maintained and increased the level of resistance to this insecticide.

2 MATERIALS AND METHODS

2.1 Location of field trials and sampling methods

The project area consisted of 22 villages, which were assigned to eight zones of three villages (see Fig. 1). The zones are physically well separated and village groups were chosen on the basis of communal mosquito breeding sites between villages within a zone, and comparable numbers of houses between zones. Our entomological data, gathered over a number of years in this area, suggest that the migration ranges of *An. albimanus* are

TABLE 1
Response of *Anopheles albimanus* to Diagnostic Concentrations of Various Insecticides in Southern Chiapas, Mexico in 1982 and 1990[a,b]

Insecticide	*Concentration* (%)	*Mortality* (%) *1982*	*1983*	*1990*
DDT	4	38	39	47
Malathion	5	84	93	99
Fenitrothion	1	44	57	99
Fenthion	2·5	97	100	
Chlorphoxim	4	98	99	100
Propoxur	0·1	89	95	
Deltamethrin	0·025	64	57	86
Cypermethrin	0·1		82	
Bendiocarb	0·1		87	
Pirimiphos-methyl	4		99	

[a] Exposure time = 1 h with the exception of fenitrothion (2 h). $n = 150–600$ insects per sample. Concentrations are World Health Organization discriminating dosages for *Anopheles*.
[b] Data taken from Reference 9.

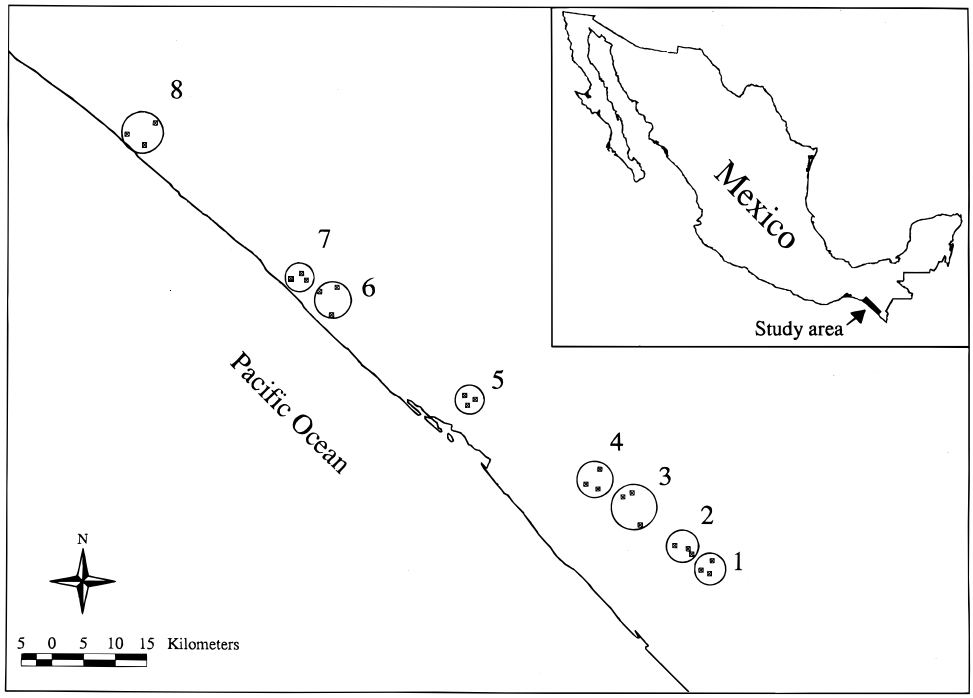

Fig. 1. Map of Southern Mexico, showing the villages within each study region where mosquitoes were collected. Study regions are separated by natural geographical barriers and no regions have any common mosquito breeding sites.

insufficient to result in significant movement of insects between treatment zones. Adult female *An. albimanus* were collected using a number of indoor and outdoor trapping methods to ensure that a large sample was collected for each zone. Mosquitoes from each trapping method were maintained separately so that we could determine whether there were any differences in resistance gene frequency from collections obtained by the different sampling methods. The treatment regimes being followed in the different zones are given in Table 2.

Collections of blood-fed females of *An. albimanus* from all 22 villages were made from February 1995 to the end of 1996. The collection methods used were indoor and outdoor human bait, cattle trap and window trap collections. The outdoor resting collections were all done in cattle yards which are in close association with the houses in these villages. As pre-exposure of some of these insects to insecticides will affect measurement of resistance gene frequencies directly on the field-caught adults, all measurements were made on the F1 progeny of these females. This also allowed us to standardise age and testing conditions of the bioassays and biochemical assays. Females were maintained under laboratory conditions until they oviposited and larvae were then reared through to adults.

TABLE 2
Insecticide Treatment Regimes followed in the Different Zones of the Study Area in Southern Mexico

Zone[a]	*Treatment*	*Insecticide*
1	Single insecticide treatment	Pyrethroid
2	Rotation	Organophosphorus (Year 1)
3	Rotation	Organophosphorus (Year 1)
4	Single insecticide treatment	DDT
5	Mosaic	Organophosphorus and pyrethroid
6	Single insecticide treatment	DDT
7	Single insecticide treatment	Pyrethroid
8	Mosaic	Organophosphorus and pyrethroid

[a] See Fig. 1.

2.2 Bioassays

All bioassays were undertake on one-day-old adult mosquitoes on insecticide-impregnated filter papers supplied by the World Health Organisation (WHO). Insects were exposed to the impregnated papers in a standard WHO test kit for 1 h, after which they were transferred to the holding chamber of the test kit and maintained for 24 h with a glucose feeder before mortalities were scored.

2.3 Laboratory insect strains

The Panama strain of *An. albimanus* has been maintained in the laboratory without insecticide selection for 20 years and prior to this was collected from an area with no history of insecticide use. This was used as the standard susceptible strain. The Mexico strain, collected from Southern Mexico in 1991, has been maintained without selection since, but still contains a low level of resistance to a number of insecticides. It is being used as a control strain to determine the rates of change in resistance gene frequencies under laboratory conditions in the absence of selection or migration.

2.4 Biochemical assays

Biochemical assays for altered acetylcholinesterase- (AChE), glutathione *S*-transferase- (GST), esterase- and monooxygenase-based resistance were undertaken as described previously.[9] Analysis of variance was used to detect any significant differences in resistance gene frequencies between the different collection zones, sexes and collection methods. Frequency distributions were plotted for each biochemical assay for the laboratory reference strains and F1s from females from different collection zones to determine the variability between populations.

2.5 DDT metabolism

Metabolism experiments were undertaken to confirm initial results from the biochemical assays. The mosquitoes from the villages with the highest GST activity were analysed for their ability to break down DDT. Batches of 47 frozen one-day-old mosquitoes from the F1 generation were assayed individually for GST activity. The remaining homogenate from each insect was stored at −20°C. The mosquitoes with the 10 highest GST activity values from each village were pooled in 10-ml glass tubes; similarly the individuals with the 10 lowest GST activity values were pooled. A further pool of 10 mosquito homogenates from each village was boiled and used as a control. The samples were spun for 10 min in a Gs-6R Centrifuge (Beckman) at 3750 rev min^{-1} at 5°C and the supernatant removed. To each supernatant was added an equal volume of sodium phosphate buffer (pH 6·5; 0·2 M) containing DDT and reduced glutathione (to final concentrations of 0·2 nM and 0·1 mM, respectively).

The mixtures were incubated at 28°C for 3 h. Extraction of DDT and its metabolites was undertaken by acidifying the reaction mixture with hydrochloric acid and extracting with chloroform (3 × 2 ml); the extracts were pooled and dried under a stream of air and stored at −20°C before analysis by HPLC as described previously.[10]

2.6 RAPD analysis of individual mosquitoes

Molecular analysis using RAPD was undertaken to look at the genetic make-up of populations sampled through different collection methods.

Genomic DNA was extracted from individual mosquitoes by adaptation of the technique of Williams *et al.*[11] Mosquitoes were homogenised in lytic buffer (Tris, pH 8·0, 200 mM; EDTA, 70 mM; NaCl, 2 M; sodium metabisulfite, 20 mM; 160 μl) and incubated at 55°C for 1 h. Sodium sarkosyl (50 g $litre^{-1}$, 40 μl) was added, the tubes inverted gently and the mixture incubated for a further 2 h. The mixture was then microfuged at 12 000*g* for 15 min and the supernatant transferred to a clean tube containing ammonium acetate (10 M; 90 μl) and isopropanol (200 μl) and the mixture microfuged at 12 000*g* for 15 min. The DNA pellet was then rinsed with ethanol + water (70 + 30 by volume), dried under vacuum, and then resuspended in Tris-HCl (pH 8·0, 10 mM; 50 μl) containing EDTA (1 mM).

The RAPD PCR reaction mixtures contained reaction buffer (KCl, 50 mM, Tris-HCl pH 9·0, 10 mM, 'Triton' X-100, 1 g $litre^{-1}$; 2·5 μl), dNTPs (2 mM; 2·5 μl), $MgCl_2$ (0·1 mM; 5 μl), Taq polymerase (0·75 Units; 0·3 μl), primer (1 μl), mosquito DNA (1 μl) made up to 25 μl with water. The reaction mixtures were put through 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min in an Omni-gene thermocycler (Hybaid) with heated lid. PCR was performed on at least three replicates of each sample.

For 10-base pair primers, OPC-01, OPC-06, OPA-07 and OPC-14 (Operon Technologies Inc, California) were used (Table 3), and the PCR products were analysed on agarose gel (14 g $litre^{-1}$). Size estimations

TABLE 3
Sequences of the Primers used for RAPD Analysis of Individual Mosquito Samples

Primer code	*5′ to 3′ sequence*
OPC-14	TGCGTGCTTG
OPA-07	GAAACGGGTG
OPC-01	TTCGAGCCAG
OPC-06	GAACGGACTC

of DNA fragments were undertaken using relative mobilities compared to a 1 kb ladder standard.

3 RESULTS AND DISCUSSION

This study has now reached the end of the pre-spray and first annual spray period. For the rotations in zones 2 and 3, an organophosphorus insecticide was used throughout the year. For the single insecticide treatments, either DDT or a pyrethroid was used, and, for the mosaic, a pattern of organophosphorus and pyrethroid insecticide, with equal numbers of houses per village being treated with each insecticide. The insecticide coverage rates were >90% in all treatment zones. In the second annual spray rounds the organophosphorus insecticide in the rotation will be replaced by a pyrethroid; the other insecticide spray regimes will remain as they were for the first year of treatment. The insecticides are being treated as class-representative chemicals, and individual trade or chemical names are not given, as the objective of this study was not to provide an endorsement for any one product within a class over other insecticides from different manufacturers.

The combined biochemical and bioassay data for the pre-spray period show that broad-spectrum resistance is still present in the *An. albimanus* field population in Southern Mexico. The resistance is conferred by a number of different resistance mechanisms which are segregating in the field population. The bioassay results using single WHO discriminating dosages for a range of insecticides (Table 4) show that, at the beginning of the study, the highest resistance frequency was to DDT, with much lower levels of resistance to carbamate and organophosphorus insecticides detected and no survivors after pyrethroid bioassays. The pattern was similar after the first year of treatment, but resistance to the pyrethroid and organophosphorus insecticides was detected at a higher level, while carbamate resistance levels were not significantly different. The highest recorded increases in pyrethroid resistance were in the single-insecticide treatment zones which had continued to be treated with DDT. It should be noted that the WHO discriminating dosages are set at double the insecticide dose that gives 100% mortality of the least susceptible *Anopheles* mosquito species. Hence these assays are good indicators of the presence of significant (<2–10-fold) resistance in a mosquito population, but they cannot be used to measure accurately resistance gene frequencies, which are likely to be higher than the bioassays suggest.

Previous studies on *An. albimanus* from Central America have shown that an altered AChE is the most common OP/carbamate resistance mechanism.[11–14] Our results suggest that this resistance mechanism is also present in the field population of *An. albimanus* from Chiapas. As expected, the range of inhibition values in the Panama susceptible laboratory strain ranged from 100% to 60%, indicating that none of the individuals in this population is carrying the altered AChE gene. In contrast, the ranges for the field-collected insects were much broader, ranging from 0% to 100% inhibition, demonstrating that the altered AChE resistance gene was present in all of the eight zones. The frequency of this resistance mechanism varied in the different field-collected samples. The frequencies of the resistance gene in the field samples are given in Table 5. The highest altered AChE gene frequencies were found in zones 1, 5 and 7, but all values were in the range 0·13 to 0·28. The altered AChE gene was still detectable in the Mexican laboratory population, but the resistance gene frequency in that colony had declined to 0·09 in the absence of pesticide selection

TABLE 4
Results of Standard World Health Organization Susceptibility Testing of F1 Mosquitoes from the Collection Zones in the Pre-spray and First Annual Post-spray Collections[ab]

	Mortality (%)							
	DDT		*Organophosphate*		*Carbamate*		*Pyrethroid*	
Collection zone	*Pre-spray*	*Post-spray*	*Pre-spray*	*Post-spray*	*Pre-spray*	*Post-spray*	*Pre-spray*	*Post-spray*
1	NT	26	100	98·9	100	100	100	99
2	28·1	NT	100	100	100	100	100	NT
3	9·4	35·7	99	98·3	100	100	100	97·6
4	46·9	21·7	100	89	100	93·5	100	87·9
5	17·4	NT	100	98·9	100	100	100	92
6	35·5	10·3	99	91	100	98·5	100	86·8
7	NT	18·4	100	89·6	95·5	100	100	97·3
8	28·9	9·4	100	100	100	86·5	99	99

[a] NT = not tested. Sample sizes are >100 for all values.
[b] Pre-spray data taken from Reference 9.

TABLE 5
Actual Resistance Gene Frequencies for the Altered AChE-based Resistance Mechanism in the Panama and Mexico Laboratory Strains and the F1 Insects from the Collection Zones in the Pre-spray Period

Strain and zone	*Resistant gene*	*Susceptible gene*	*n*
Panama	0	1·00	94
Mexico	0·09	0·91	102
1	0·23	0·77	291
2	0·13	0·87	272
3	0·21	0·79	205
4	0·19	0·81	114
5	0·28	0·72	368
6	0·21	0·79	126
7	0·24	0·76	142
8	0·16	0·84	265

pressure in the laboratory over a number of years.

We showed earlier that there were significant differences in the gene frequencies of this resistance mechanism between different mosquito collection methods.[9] Hence mosquitoes obtained by different collection methods were analysed separately in the first post-spray year. Frequencies of this resistance mechanism have generally declined marginally in all zones in this year compared to the pre-spray year.

3.1 Elevated esterase-based resistance

There was no evidence of any elevated esterase-based resistance mechanism in the *An. albimanus* field populations with α- or β-naphthyl acetate, either in the pre-spray period or after the first three rounds of spraying. The mean values for *p*-nitrophenyl acetate activity of one-day-old *An. albimanus* in all the collection zones were 2·5–3-fold higher than those for the susceptible strain although all values were still significantly lower than the rates seen in *Culex* mosquitoes with elevated activity due to amplified resistance-associated esterases.[15]

3.2 Monooxygenases-based resistance

The Panama susceptible laboratory strain was homogeneous for low level cytochrome P450 content as measured by haem titration. There were a small number of individuals from the F1 generation of the females from the collection zones with elevated levels of monooxygenases. The role of these monooxygenases in OP and/or pyrethroid resistance is currently being determined by metabolism studies.

3.3 Glutathione-*S*-transferase (GST)-based resistance

Elevated GST is a major mechanism of DDT resistance in *Anopheles* mosquitoes and a cause of both DDT and OP resistance in houseflies and *An. subpictus* Grassi.[16–20] The resistance mechanism can be detected by assaying the total GST activity of the insect with the general GST substrate chlorodinitrobenzene (CDNB). Once elevated activity levels are detected, the correlation with resistance needs to be confirmed by metabolism studies on the relevant insecticides. There was evidence of a GST-based resistance mechanism in the field collections of *An. albimanus*. Analysis of the data from this period also showed that GST activities of one-day *An. albimanus* (F1 generation) were significantly different between the collection zones ($P < 0{\cdot}01$); GST activity averages ranged from 0·1890(±0·1403) to 0·6774(±1·5370) mmol min^{-1} mg^{-1} protein. Average activity values for all collection zones were higher than for the laboratory susceptible Panama strain (0·1671(±0·0421)). The highest average GST activity was in zone 3, which had four-fold higher activity than the Panama strain (Table 6). This order of magnitude of change in GST activity is similar to that seen in *An. gambiae* Giles with GST-based DDT resistance.[18,21] There was significant variability of GST activity for progeny of females obtained by different collection methods (Table 7).

3.4 DDT metabolism

Results of DDT metabolism of mosquitoes from the F1 generation collected from zones 1 and 5 are shown in Table 8. Individual insect homogenates were checked

TABLE 6
One-Day-Old *Anopheles albimanus* GST Activity in the Panama and Mexico Laboratory Strains and F1 Generation from the Collection Zones in the Pre-spray Period[a]

Strain and zones	*GST activity mmol min^{-1} mg^{-1} protein (± SD)*	*n*	*Panama strain*
Panama	0·17(±0·04)	94	
Mexico	0·20(±0·19)	103	1·2
1	0·45(±0·49)	589	2·7
2	0·35(±0·34)	264	2·1
3	0·68(±1·54)	188	4
4	0·20(±0·17)	95	1·2
5	0·45(+0·43)	690	2·7
6	0·19(±0·14)	123	1·1
7	0·37(±0·62)	135	2·2
8	0·29(±0·27)	375	1·7

[a] From Reference 9.

TABLE 7
Comparisons of One-Day-Old *Anopheles albimanus* GST Activity between the Collection Method in the Collection Zones[a]

Collection zone	*Human bait outdoor*	*n*	*Human bait indoor*	*n*	*Corral*	*n*	*Curtain trap*	*n*	$P < 0{\cdot}001$
1	0·18 (±0·09)	174	0·46 (±0·4)	225	0·70 (±0·66)	190			*
2					0·35 (±0·35)	264			
3	0·35 (±0·77)	25	1·87 (±3·15)	37	0·39 (±0·19)	126			*
4	0·16 (±0·14)	11	0·16 (±0·11)	16	0·21 (±0·18)	68			
5	0·48 (±0·14)	22	0·53 (±0·52)	424	0·30 (±0·14)	192	0·35 (±0·16)	52	*
6	0·15 (±0·07)	73	0·18 (±0·11)	13	0·28 (±0·20)	37			*
7	0·4 (±0·42)	15	0·26 (±0·08)	96	0·80 (±1·38)	24			*
8	0·10 (±0·06)	28	0·19 (±0·13)	5	0·31 (±0·28)	328	0·08 (±0·06)	14	*
Total	0·21 (±0·26)	348	0·53 (±0·85)	816	0·39 (±0·43)	1229	0·30 (±0·18)	66	*

[a] Means (±SD) mmol min^{-1} mg^{-1} protein.

initially for GST activity levels and subsequently pooled in high- and low-GST activity groups. After correction for DDT degradation occurring in boiled control insects, there was no evidence of any metabolism of DDT in the low-GST activity group. In contrast, the DDT metabolite, DDE, was found in significant quantities in both the high-GST activity groups, confirming that the high GST levels detected in the field population of *An. albimanus* are associated with DDT resistance.

3.5 RAPD analysis of the genetics of the population structure

As a number of the biochemical assays had indicated that significant differences in gene frequencies occurred in the progeny of females collected from the field by different sampling methods, RAPD analysis was undertaken to determine whether there was any evidence of different species or sub-populations of *An. albimanus* being sampled by different collection methods. Individual DNA fragments were scored as being present or absent on agarose gels for each of the four primers. Only clear DNA bands were scored. Of the four primers, only OPC-01 produced any bands that differed between individuals. However, analysis of 30 individual mosquitoes from different villages obtained by different collection methods revealed no statistical difference between mosquitoes collected in different villages and by different collection methods. The remaining three primers gave complex patterns of bands which were identical between different village and sampling collections. It was therefore concluded that at the level of resolution of RAPDs we were able to find no evidence of different collection methods representing a genetically distinct sub-set of the population. We are currently investigating the use of microsatellites to determine whether the mosquitoes we are obtaining by different collection methods can be considered as samples of the same population.

TABLE 8
DDT Metabolism by One-Day-Old *Anopheles albimanus* from Collection Zones 1 and 5 separated on the Basis of High or Low GST Activity.[a]

Collection zone	*Metabolites*	*High activity* (%)	*Low activity* (%)
1	DDT	89·4	100
	Dicofol	0	0
	DDD	0	0
	DDE	10·5	0
	Recovery rates	55	69·4
5	DDT	71·4	99·7
	Dicofol	0	0·3
	DDD	0	0
	DDE	28·6	0
	Recovery rates	55·2	47·4

[a] From Reference 9.

ACKNOWLEDGEMENTS

This work was sponsored by the Insecticide Resistance Action Committee (IRAC; Public Health Section), with further contributions from Agrevo, Bayer, Cheminova, FMC, Mitsui Toatsu, Rhône Poulenc, Sumitomo and Zeneca.

REFERENCES

1. Curtis, C. F., Hill, N. & Kasim, S. H., Are there effective resistance management strategies for vectors of human disease? *Biol. J. Linn. Soc.*, **48** (1993) 3–18.
2. Mellon, R. B. & Georghiou, G. P., Rotational use of insecticides in mosquito control programmes. *Proc 52nd Ann. Calif. Mosq. Cont. Assoc.* (1984) 65–7.
3. Georghiou, G. P., Insecticide resistance and prospects for its management. *Res. Rev.*, **76** (1980) 131–44.
4. Greever, J. & Georghiou, G. P., Computer simulation of control strategies for *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol.*, **16** (1979) 180–8.
5. Curtis, C. F., Theoretical models of the use of insecticide mixtures for the management of resistance. *Bull. Ent. Res.*, **75** (1985) 259–65.
6. Tabashnik, B. E., Managing resistance with multiple pesticide tactics: Theory, evidence and recommendations. *J. Econ. Entomol.*, **82** (1989) 1263–9.
7. Taylor, C. E., Quaglia, F. & Georghiou, G. P., Evolution of resistance to insecticides: a cage study on the influence of migration and insecticide decay rates. *J. Econ. Entomol.*, **76** (1983) 704–6.
8. Hemingway, J., A note on simple biochemical methods for resistance detection and their field application in Sri Lanka. *Pestic. Sci.*, **27** (1989) 281–5.
9. Penilla, R. P., Rodriguez, A. D., Hemingway, J., Estrada, J. L. T., Jimenez, J. I. A. & Rodriguez, M. H., Rotational and mosaic strategies for delaying the development of insecticide resistance in mosquitoes—baseline data for a large scale field trial in Southern Mexico. In *Proc. 2nd Internat. Conf. Insect Pests in the Urban Environment*, ed. K. B. Wildey & W. H. Robinson. BPCC Wheatons Ltd, Exeter, UK, 1996 pp. 401–11.
10. Prapanthadara, L., Hemingway, J. & Ketterman, A. J., DDT-resistance in *Anopheles gambiae* Giles from Zanzibar Tanzania, based on increased DDT-dehydrochlorinase activity of glutathione *S*-transferases. *Bull. Entomol. Res.*, **85** (1995) 267–74.
11. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.*, **18** (1990) 6531–5.
12. Ayad, H. & Georghiou, G. P., Resistance to organophosphates and carbamates in *Anopheles albimanus* based on reduced sensitivity to acetylcholinesterase. *J. Econ. Entomol.*, **68** (1975) 295–7.
13. Georghiou, G. P. Studies of resistance to carbamate and organophosphorus insecticides in *Anopheles albimanus*. *Am. J. Trop. Med. Hyg.*, **21** (1972) 797–806.
14. Hemingway, J. & Georghiou, G. P., Studies on the acetylcholinesterase of *Anopheles albimanus* resistant and susceptible to organophosphate and carbamate insecticides. *Pestic. Biochem. Physiol.*, **19** (1983) 167–71.
15. Karunaratne, S. H. P. P., Hemingway, J., Jayawardena, K. G. I., Dassanayaka, V. & Vaughan, A., Kinetic and molecular differences in the amplified and non-amplified esterases from insecticide resistant and susceptible *Culex quinquefasciatus* mosquitoes. *J. Biol. Chem.*, **270** (1995) 31124–8.
16. Hemingway, J., Miyamoto, J. & Herath, P. R. J., A possible novel link between organophosphorus and DDT insecticide resistance genes in *Anopheles:* supporting evidence from fenitrothion metabolism studies. *Pestic. Biochem. Physiol.*, **39** (1991) 49–56.
17. Hemingway, J., Jayawardena, K. G. I., Weerasinghe, I. & Herath, P. R. J., The use of biochemical tests to identify multiple insecticide resistance mechanisms in field-selected populations of *Anopheles subpictus* Grassi (Diptera: Culicidae). *Bull. Entomol. Res.*, **77** (1987) 57–66.
18. Prapanthadara, L. A., Kuttastep, S., Hemingway, J. & Ketterman, A. J., Characterization of the major form of glutathione transferase in the mosquito *Anopheles dirus* A. *Biochem. Soc. Trans.*, **23** (1995) 81S.
19. Clark, A. G., Shamaan, N. A., Dauterman, W. C. & Hayaoka, T., Characterization of multiple glutathione transferases from the housefly, *Musca domestica* (L). *Pestic. Biochem. Physiol.*, **22** (1984) 51–9.
20. Clark, A. G. & Shamaan, N. A., Evidence that DDT-dehydrochlorinase from the house fly is a glutathione *S*-transferase. *Pestic. Biochem. Physiol.*, **22** (1984) 249–61.
21. Hemingway, J., Malcolm, C. A., Kissoon, K. E., Boddington, R. G., Curtis, C. F. & Hill, N., The biochemistry of insecticide resistance in *Anopheles sacharovi:* comparative studies with a range of insecticide-susceptible and -resistant *Anopheles* and *Culex* species. *Pestic. Biochem. Physiol.*, **24** (1985) 68–76.